Journal of Forensic and Legal Medicine 20 (2013) 211–216

Contents lists available at SciVerse ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Original communication

Postmortem vitreous chemistry – An evaluation of sodium, potassium and chloride levels in estimation of time since death (during the first 36 h after death)

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ARTICLE INFO

Article history:
Received 26 March 2012
Received in revised form
29 June 2012
Accepted 2 September 2012
Available online 21 September 2012

Keywords:
Vitreous chemistry
Postmortem
Time since death
Postmortem interval
Sodium, potassium and chloride levels

ABSTRACT

Estimation of time since death is a paramount medico-legal issue in any postmortem examination. The present study is intended to study the correlation between postmortem interval and vitreous humor chemistry for sodium, potassium, and chlorides. The study is aimed to find male–female differences and differences between right and left eyes in vitreous chemistry. The vitreous humor samples were collected in 114 autopsies conducted in the study center and analyzed biochemically. All the cases where exact time of death was known and where the time since death ranged between 0 and 36 h were included in the study. Data obtained was analyzed statistically using spss version 11.0. The present research did not find a significant correlation between vitreous chemistry and postmortem interval. The differences in vitreous sodium, potassium, chloride levels and the sodium potassium ratio among males and females and between right and left eyes were not found to be statistically significant.

1. Introduction

Estimation of time since death is a paramount medico-legal issue in any postmortem examination. Postmortem interval (PMI) is the time elapsed between death of a person and the time of autopsy. Though the exact time of death can rarely be estimated on the basis of autopsy findings alone, an appropriate range of PMI can be deduced by intelligent interpretation of various changes that take place after death. Body fluids like pericardial fluid, cerebrospinal fluid, aqueous and vitreous humor, and synovial fluid show postmortem alterations in the levels of their electrolytes. These changes usually progress in a well designated manner until putrefaction sets in and can help forensic pathologist to estimate time since death (TSD) with a reasonable accuracy.[1] These body fluids are located in closed compartments and thus, are not contaminated quickly after death.[2]

Vitreous humor is the most investigated body fluid for estimation of postmortem interval from chemical changes taking place in its constituent electrolytes after death. Vitreous humor is a fairly stable fluid in the postmortem period and is hardly contaminated even in the late postmortem interval.[3] Its isolated topography, compared to blood and CSF and its resistance to microbiological contamination with bacterial degradation makes vitreous humor a very suitable medium for postmortem biochemical investigation. The biochemical analytes of vitreous humor like potassium, sodium, chloride, calcium, magnesium, phosphate, urea, creatinine and lactate have been analyzed to estimate the postmortem interval.[4]

The present study is intended to study the correlation between postmortem interval and vitreous humor chemistry for sodium, potassium, and chlorides. The study is aimed to find male–female differences and differences between right and left eyes in vitreous chemistry.

2. Material and methods

The vitreous humor samples were collected in 114 medicolegal autopsies conducted in the department of Forensic Medicine, JSS Medical College (JSS University) and Mysore Medical College and Research Institute, Mysore, India. A prior approval was

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http://dx.doi.org/10.1016/j.jflm.2012.09.001

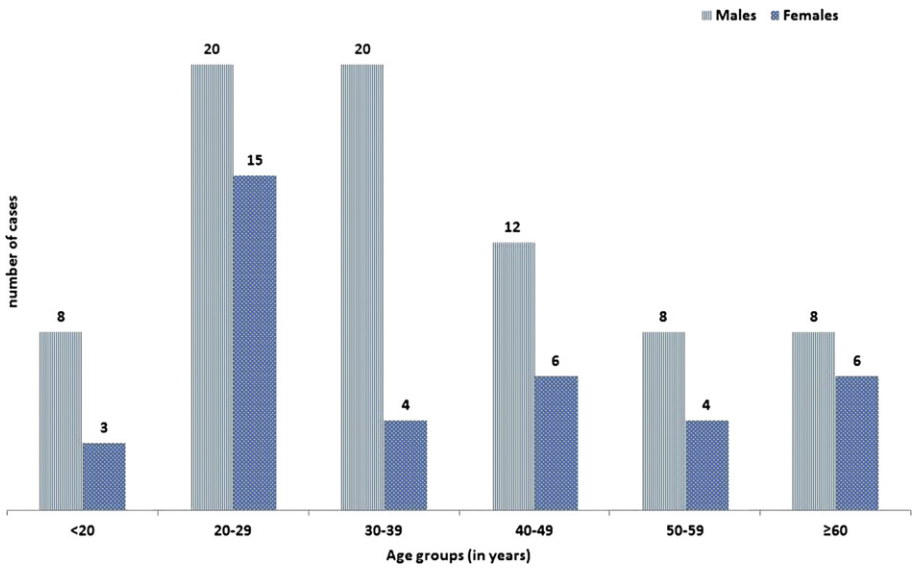

Fig. 1. Age distribution of cases included in the study.

obtained from the Institutional Ethical Committee to conduct this research.

2.1. Characteristics of cases

The cases included in the study were aged between 12 and 77 years with a mean age of 36.4 ± 15.6 years. Two-third of the cases were males ($n = 76$) and remaining females ($n = 38$), male–female ratio of the cases included in the study being 2: 1. Sex-wise age distribution of cases is shown in Fig. 1. Traumatic deaths comprised of the majority of cases ($n = 62$) followed by fatal poisonings ($n = 26$) and asphyxia deaths ($n = 20$). Four cases were designated as deaths from natural causes, while the remaining two cases were of snake and scorpion bite envenomation respectively.

All the cases where exact time of death was known and where the time since death ranged between 0 and 36 h were included in the study. The precise time of death was noted in all cases. Cases with known or suspected ocular disease, localized trauma, head injury involving orbit and in cases where the vitreous was contaminated with blood and tissues were not included in the study. The vitreous humor samples were collected from both eyes by the method proposed by Coe.[5]

2.2. Instrumentation, sampling and pre-analysis treatment

Vitreous was collected from right and left eyes separately using a sterilized 20 gauge hypodermic needle. Around 2 ml crystal clear vitreous humor was aspirated through a scleral puncture on the lateral canthus of each eye. Clear fluid thus obtained was analyzed for the study and samples contaminated with tissue fragments were discarded. After drawing the vitreous humor, 2 ml of normal saline was injected in each eye for cosmetic restoration of eyeball.

Samples were centrifuged at 4500 rpm and supernatant fluid was transferred to another container and analysis was done in the department of Biochemistry. The analysis was carried out immediately after extraction of vitreous for the sodium, potassium, and chloride levels. Care was ensured to prevent any contamination with blood/RBC's. In the laboratory vitreous electrolytes (sodium, potassium and chloride) were estimated by Ion selective electrode method on Roche 9180 Electrolyte Analyzer. Electrodes were fitted with liquid ion exchange membranes that incorporate valinomycin. Daily quality checks were performed using level 2 and level 3 quality control sera.

2.3. Data analysis

All the samples were classified into four groups based on time since death to study the postmortem alterations in the vitreous sodium, potassium and chloride levels. Group I included cases with postmortem interval of 0–6 h, group II included those with postmortem interval of 6–12 h, group III with a postmortem interval of 12–18 h, and group IV included cases with postmortem interval of >18 h. Data was analyzed using spss version 11.0. Comparative biochemical analysis for vitreous humor between right and left eyes and between males and females was done using Student's *t*-test. Pearson's correlation was used to study the correlation between time since death and vitreous chemistry for sodium, potassium, and chloride and sodium-potassium ratio. Level of significance was set at p-value <0.05.

3. Results

Mean vitreous sodium, potassium, chloride levels and the sodium potassium ratio in right and left eyes are shown in Table 1. Vitreous sodium and chloride levels were higher in the left eye while vitreous potassium levels were higher in the right eye. The sodium potassium ratio in vitreous was observed to be higher in the left eye. The differences in vitreous sodium, potassium, chloride levels and the sodium potassium ratio between right and left eyes however were statistically not significant (Table 1). Owing to the non-significant differences of vitreous sodium, potassium, chloride

Table 1
Vitreous thanatochemistry in right and left eyes.

	Right ($n = 114$)	Left ($n = 114$)	*t*-value	*p*-value
	Mean (S.D)	Mean (S.D)		
Na^+ (MEq/L)	134.96 (24.11)	135.19 (23.56)	−0.115	0.909
K^+ (MEq/L)	9.16 (2.82)	9.01 (2.82)	0.660	0.511
Cl^- (MEq/L)	120.15 (21.24)	120.71 (22.12)	−0.321	0.749
Na^+ / K^+	15.69 (4.04)	16.07 (4.33)	−1.159	0.249

S.D. – Standard Deviation.

Table 2
Descriptive statistics – vitreous thanatochemistry and time since death ($n = 114$).

	Na^+ (MEq/L)	K^+ (MEq/L)	Cl^- (MEq/L)	Na^+/K^+	TSD (hours)
Minimum	71.50	3.00	64.50	8.58	0.08
Maximum	194.50	16.60	170.00	27.72	36.00
Mean	135.08	9.09	120.43	15.72	12.51
S.D.	21.35	2.56	19.50	3.75	6.77
Median	134.25	8.78	118.50	15.52	12.54

S.D. – standard deviation, TSD – time since death.

levels and the sodium potassium ratio between right and left eyes, mean values of right and left eyes were derived and used for further analysis.

Time since death (TSD) in the study sample ranged between 0.08 h (5 min) and 36 h. Mean TSD in the study was observed to be 12.51 h. Vitreous sodium levels ranged between 71.50 and 194.50 MEq/l, potassium levels between 3.00 and 16.60 MEq/l and chloride levels between 64.50 and 170.00 MEq/l. The sodium potassium ratio in vitreous humor ranged between 8.58 and 27.72. The mean vitreous sodium, potassium, chloride levels and the sodium potassium ratio was 135.08 MEq/l, 9.09 MEq/l, 120.43 MEq/l, and 15.72 respectively. Descriptive statistics for vitreous chemistry and time since death are shown in Table 2.

Mean time since death among males and females was 12.76 h and 12.03 h respectively. The male–female differences in time since death were statistically insignificant. Comparative analysis of male and female values for vitreous sodium, potassium, chloride levels and the sodium potassium ratio are depicted in Table 3. It is observed that the vitreous sodium and chloride levels were higher in males while vitreous potassium levels were higher in females. The sodium potassium ratio in vitreous was observed to be higher in males. The male–female differences in vitreous sodium, potassium, chloride levels and the sodium potassium ratio however were statistically not significant (Table 3). Mean values for vitreous electrolytes and time since death in different age groups is shown in Table 4. Correlation between age and vitreous electrolytes was not found to be significant. Pearson's correlation coefficient (r) for correlation between age and Sodium, Potassium, Chloride and sodium potassium ratio was -0.025 ($p = 0.788$), -0.034 ($p = 0.718$), -0.047 ($p = 0.617$), and 0.057 ($p = 0.547$) respectively.

Concentration of vitreous sodium and chloride show a marginal fall with increase in time since death (Fig. 2) while vitreous potassium shows a marginal rise with increase in time since death (Fig. 3). Pearson's correlation between time since death and vitreous sodium, potassium, chloride levels and the sodium potassium ratio was obtained for different age groups and overall for the total sample. The correlation between vitreous electrolytes and time since death was not found to be statistically significant (Table 5). All the cases were classified into four groups based on time since death (<6 h, 6–12 h, 12–18 h, >18 h). Mean vitreous sodium, potassium, chloride levels and the sodium potassium ratio did not show any specific trend among these four groups based on time since death (Table 6). Vitreous potassium was raised throughout in all the four groups, while sodium and chloride were

Table 3
Male–female differences in vitreous thanatochemistry and time since death.

	Male ($n = 76$)	Female ($n = 38$)	t-value	p-value
	Mean (S.D)	Mean (S.D)		
Na^+ (MEq/L)	136.38 (20.04)	132.47 (23.84)	0.921	0.359
K^+ (MEq/L)	9.08 (2.60)	9.10 (2.52)	−0.041	0.967
Cl^- (MEq/L)	121.62 (18.37)	118.05 (21.65)	0.921	0.359
Na^+/K^+	15.99 (3.96)	15.17 (3.28)	1.091	0.278
TSD (hours)	12.76 (6.64)	12.03 (7.08)	0.567	0.592

S.D. – standard deviation, TSD – time since death.

Table 4
Mean values for vitreous electrolytes and time since death in different age groups.

Age groups	n	Na^+ (MEq/L)	K^+ (MEq/L)	Cl^- (MEq/L)	Na^+/K^+	TSD (hours)
<20 years	11	138.10	8.90	121.60	16.10	11.90
20–29 years	35	137.20	9.30	122.80	15. 40	12.00
30–39 years	24	131.90	9.10	118.70	15.30	12.30
40–49 years	18	129.90	8.60	116.80	15.90	11.70
50–59 years	12	139.30	9.40	121.60	15.60	13.50
≥60 years	14	135.8	8.90	120.30	16.60	14.60

n – number of cases, TSD – time since death.

in their normal ranges. Only in two cases vitreous potassium was observed to be in the normal ranges. Other cases showed a raised potassium levels soon after death (Fig. 3).

4. Discussion

Accurate prediction of time of death is of great value in medicolegal investigations. Previous studies have reported the possibility of accurate prediction of time since death based on the alterations in the levels of vitreous electrolytes and corresponding equations and formulas have been reported in literature to precisely estimate the postmortem interval.

The differences in vitreous sodium, potassium, chloride levels and the sodium potassium ratio between right and left eyes in the present study were statistically not significant. Our findings are in accordance with that of Mulla et al.[6] who evaluated the between-eye differences for vitreous humor biochemical constituents and did not find any significant differences in vitreous chemistry between eyes at identical postmortem interval. Similar observations are made for between eye differences in vitreous potassium levels in Myanmar and Japanese populations.[7] Pounder et al.[8] however, have suggested significant differences for vitreous potassium concentrations between the eyes of the same individual.

The present research did not observe any statistically significant male–female differences in the vitreous sodium, potassium, and chloride concentrations and in the sodium potassium ratio. Likewise no age related changes were observed in vitreous electrolytes for the cases included in the study. The correlation between age and vitreous electrolytes was statistically not significant. Our observations are similar to studies in Indian, Myanmar and Japanese populations where no significant age and sex differences were observed for vitreous potassium levels.[1,7] Jashnani et al. in a recent study from India[9] concluded that age, sex, cause of death, season of death and refrigeration of sample does not influence vitreous potassium levels. Coe[5] however, suggested that the age of an individual may have some effect on vitreous potassium. Blumfield et al.[10] in a study on postmortem vitreous chemistry in childhood deaths suggested that the age at death affected the rate of increase of vitreous potassium after death and the regression slope was steeper for children than for adults.[11]

In the present research the concentration of vitreous sodium and chloride show a marginal fall with increase in time since death and vitreous potassium shows a marginal rise with increase in time since death. The correlation between vitreous electrolytes and time since death however, was not found to be statistically significant. Mean vitreous sodium, potassium, chloride levels and the sodium potassium ratio did not show any specific trend in relation to time since death. Postmortem vitreous chemistry is largely studied by various researchers.[12] Vitreous sodium and chloride levels have been observed to be relatively stable after death during the early postmortem period and may be useful in determination of the mechanism of death.[13] It is possible to diagnose hyponatremia or hypernatremia at the time of death as the abnormalities in

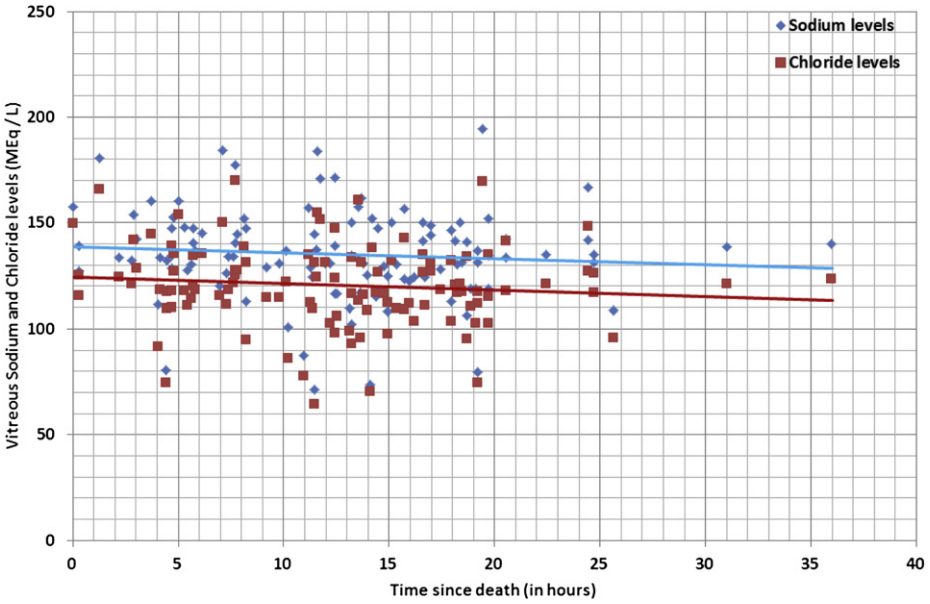

Fig. 2. Scatter diagram for postmortem sodium and chloride levels and time since death.

antemortem serum sodium and chloride concentrations are reflected in postmortem vitreous values.[14] Jashnani et al.[9] did not find any correlation between vitreous sodium and chloride levels with postmortem interval. Observations of the present investigations with regard to vitreous sodium and chloride levels are similar to that reported by earlier researchers.[9,15] Vitreous electrolytes such as sodium, chloride, creatinine and lactate remain stable in their concentrations when analyzed in postmortem samples while other analytes show considerable changes in their concentrations. The more stable parameters are better suited for detection of antemortem metabolic changes, whereas alterations in concentration of unstable analytes are utilized in estimation of postmortem interval.[16] Of all the vitreous constituents, vitreous potassium is the most extensively studied parameter for estimation of time since death. Different statistical approaches for an accurate estimation of the time since death from vitreous potassium concentration have been proposed.[17] Many of the statistical models and equations derived to estimate time since death are based on the assumption that the postmortem increase in vitreous potassium is fairly linear with time and changes at a constant rate.[9] Previous studies on the correlation of vitreous potassium level and time since death have established a linear increase in vitreous potassium with postmortem interval.[1,7,18–20] The relationship between vitreous potassium and postmortem interval however, is not found to be entirely linear.[11]

The practical utility of vitreous potassium levels in estimation of time since death accurately is debated time and again. Review of the literature revealed an extensive disagreement on the usefulness

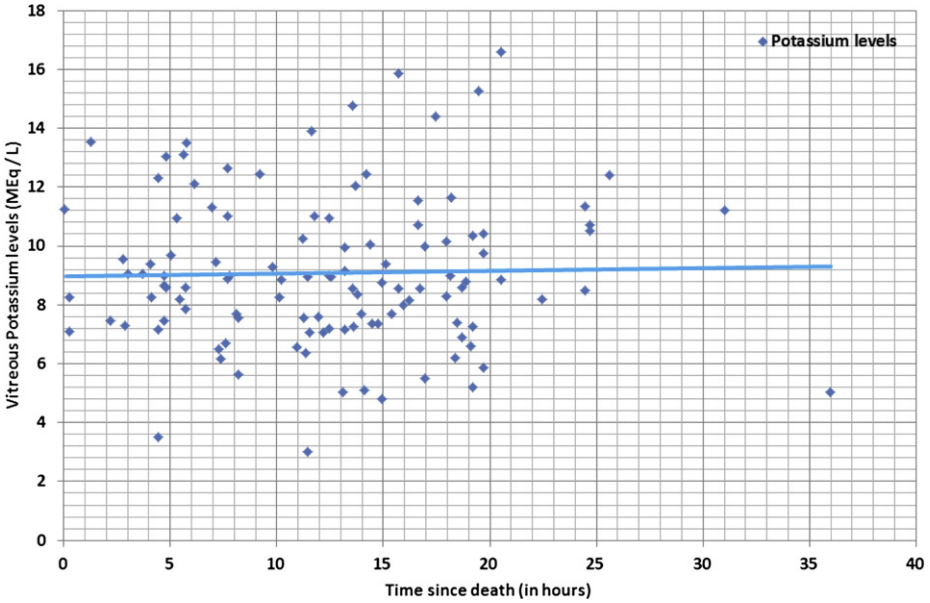

Fig. 3. Scatter diagram for postmortem potassium levels and time since death.

Table 5
Correlation (r) between time since death and vitreous thanatochemistry in different age groups and overall for the total sample.

TSD	Na^+ (MEq/L)	K^+ (MEq/L)	Cl^- (MEq/L)	Na^+/K^+
Age groups	r (p-value)	r (p-value)	r (p-value)	r (p-value)
<20 years	0.290 (0.387)	0.474 (0.141)	0.264 (0.432)	−0.324 (0.332)
20–29 years	−0.003 (0.968)	−0.159 (0.361)	−0.009 (0.958)	0.225 (0.194)
30–39 years	−0.381 (0.066)	−0.064 (0.768)	−0.383 (0.065)	−0.098 (0.650)
40–49 years	−0.155 (0.538)	0.435 (0.071)	−0.227 (0.365)	−0.691 (0.002)
50–59 years	−0.019 (0.952)	0.045 (0.889)	−0.121 (0.707)	−0.066 (0.838)
≥ 60 years	−0.127 (0.665)	−0.220 (0.450)	−0.057 (0.847)	0.424 (0.130)
Overall	−0.087 (0.360)	0.023 (0.808)	−0.108 (0.253)	−0.026 (0.785)

TSD – time since death (hours), r – pearson correlation.

of vitreous potassium concentration as a predictor of the postmortem interval.[21] Most studies have shown that it can be a useful tool in estimating time since death during the early postmortem period. There however, is no agreement on the duration till when vitreous potassium can be considered a reliable criterion for estimation of time since death and on its precision in estimating time since death. The present investigation did not find any significant correlation between vitreous potassium and time since death. Adjutantis and Coutselinis[22] reported a possibility of accurate prediction of time since death (within 2 h) from the estimation of potassium in the vitreous humor. Leahy and Farber[16] did not find any mathematical relationship between vitreous potassium and postmortem interval in cases of sudden death. Forensic scientists are of the opinion that vitreous potassium should not be the definitive method of choice for estimation of postmortem interval but used selectively and in conjunction with other tests.[11]

Problems with the accuracy and precision of measurements in vitreous chemistry are reported in previous studies.[23–25] The main reason for the differences in vitreous chemistry and its relation with postmortem interval among different studies and conflicting reports about between eye differences at identical postmortem interval may be attributed to variation in study methods. The levels may vary based on different analytical procedures and instruments used.[26] The variation in values of vitreous humor electrolytes may in addition be related to the composition of vitreous humor and the preanalytical handling.[27] An obvious discrepancy may be in the aspiration technique adopted by some investigators. Bito[28] reported that concentration of many solutes in the vitreous humor are different in anterior and posterior vitreous chambers and also suggested that the concentrations of vitreous solutes next to the retina is different than the concentration in the central portion of the globe and therefore is essential to aspirate vitreous humor as completely as possible to reflect accurately the concentration levels of all solutes. Henssge and Madea[29] suggested that many internal and external, and antemortem and postmortem factors influence the characteristics of the curve and the start point. These influencing factors thus, need to be studied quantitatively in order to improve the precision of time since death estimation. Henssge and Madea were critical of the research on estimation of time since death and concluded that the amount of literature on estimating the time since death was of only academic interest and had a reverse correlation with its importance in practice.[29] Madea suggested a need to develop analytical methods optimized just for vitreous humor for precise measurements.[4]

Table 6
Variations in vitreous thanatochemistry in relation to time since death.

TSD (hours)	<6 (n = 27)	6–12 (n = 31)	12–18 (n = 38)	>18 (n = 18)
	Mean (S.D)	Mean (S.D)	Mean (S.D)	Mean (S.D)
Na^+ (MEq/L)	139.06 (18.74)	135.76 (25.62)	131.46 (18.34)	135.58 (23.41)
K^+ (MEq/L)	9.40 (2.39)	8.60 (2.33)	8.98 (2.58)	9.67 (3.16)
Cl^- (MEq/L)	124.57 (18.74)	120.68 (22.69)	117.29 (16.57)	120.44 (20.88)
Na^+ / K^+	15.53 (3.39)	16.47 (3.47)	15.50 (3.88)	15.14 (4.52)

S.D. – standard deviation, TSD – time since death.

5. Conclusions

- The present research did not find any correlation between postmortem interval and vitreous humor chemistry for sodium, potassium, and chlorides and concludes that its role alone in estimation of time since death is limited.
- The differences in vitreous sodium, potassium, chloride levels and sodium potassium ratio between right and left eyes were not found to be statistically significant in the present research.
- The present research did not observe any statistically significant male–female differences in vitreous sodium, potassium, chloride levels and in the sodium potassium ratio.

Limitations in the present study exist primarily due to the fact that the present research comprises of a low number of cases and that no ante-mortem biochemical data were available for the cases included in the study. Future studies on correlation between vitreous electrolytes and time since death on a larger sample size are hence, proposed.

Ethical approval

A prior approval was obtained from the Institutional Ethical Committee of JSS Medical College, Mysore, India.

Funding

No support in the form of grants.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgment

We wish to acknowledge the anonymous reviewers for the very useful suggestions during the revision of the article

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